Final Report on Investigations of Fungus Agents Pathogenic to Rice.

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FINAL REPORT ON INVESTIGATIONS OF FUNGOUS AGENTS PATHOGENIC TO RICE, WITH SUPPLEMENTARY NOTES ON FACTORS AFFECTING THE PATHOGENICITY OF SCLEROTIUM ROLFSII

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In the preliminary report, submitted March 27, 1944, on agents pathogenic to rice, emphasis was placed on two aspects of the general problem:

(1) Fungi capable of damaging rice plants in the seedling and juvenile stages previous to the transplanting stage. Pp. 2-13.

(2) Combination inoculations with two or more pathogens to determine their compatibility and their capacity to produce disease complexes of increased severity. Pp. 16-18.

A further report is now presented on these two subjects and a summary is given of studies on several miscellaneous topics relating to the general project, which are itemized as follows:

(3) Inoculation technique with (a) seedlings and (b) plants growing with submerged roots. Pp. 19, 22-23

(4) Measurement of plant injury resulting from infection. Pp.19-20.

(5) Comparative infectivity of different strains of Helminthosporium oryzae in relation to conidial vs. mycelial growth habit. Pp.21-23.

(6) Comparative infectivity of different strains of Rhizoctonia spp. for rice. P. 24

(7) Comparison of growth and infectivity of inoculum consisting of natural seed vs. artificial pellets. Pp. 25-26

(8) Comparative infectivity of different strains of Rhizoctonia alone and in combination with Sclerotium rolfsii for beans. P. 26

(9) Summary and conclusions. P. 27

1. Factors influencing infection by rice seedling blight fungi

In preliminary tests none of the fungi named below were able to cause mortality of rice seedlings growing in a greenhouse under representative seedbed conditions, when inoculation was delayed until the plants were as much as 6 inches high, and when the inoculum was placed on the soil surface in contact with the base of the stem. Cultures on various materials, including bran and the following seeds -- millet, sorghum, buckwheat, cowpea and lupin--on all of which vigorous growth was obtained, were tried. The fungi tested were Helminthosporium oryzae (HO), several strains including conidial and mycelial type; Piricularia oryzae (PO); Fusarium moniliforme (FM), isolated from rice seed; Rhizoctonia solani (RS), 20 strains obtained from a variety of host plants and localities; Sclerotium rolfsii,* including a strain from rice and others from different hosts. The following rice varieties were tested: Acadia, Blue Rose, Caloro, Rexoro, Fataribune, and a Chinese variety of unknown identity.

In further tests, in which rice plants grown singly and also up to 5 plants in a 3 inch pot (to determine the effect of crowding), were inoculated at various stages from 6 to 12 inches high, and were then held in moist chambers at various temperatures until the fungi had grown extensively over the soil surface and on the plant, they still rarely succumbed to infection although a basal sheath rot of variable severity developed.

It was therefore evident that, in order to cause appreciable damage by those fungi, exposure to infection would have to occur at (a) an earlier stage of growth, (b) under environmental conditions less favorable for the host, or (c) to inoculum of greater virulence. The question also was raised whether the more or less superficial sheath rot that resulted at times from these inoculations would cause a material reduction of growth or loss of crop at a later stage. Investigations on the relations of these factors to infection and crop damage are summarized in the following:

A. Effect of stage of growth and position of inoculum.

Flats of 1 square foot area containing steamed soil were inoculated with each of the fungi listed below, using 100 seeds of a sorghum culture as inoculum, which were mixed into the soil to a depth of 1/2 inch. 25 rice seedlings with sprouts 1 to 2 inches long were set in each flat, and the flats were thereafter kept under ordinary greenhouse care. After 7 weeks of growth, with the plants then 15 to 18 inches tall, the results as to stand and condition were:

* Sclerotium rolfsii is designated CO.

Inoculation	Per cent of Plants surviving	Per cent of normal plants.
HO (W Strain)	84	72
PO	76	72
PW	88	76
RS (Strain 341)	92	68
CO (rice strain)	68	44
none	92	88

Even with this extremely heavy inoculation nearly 75 per cent of the plants survived and remained apparently normal except in the flat inoculated with CO where the percentage of normal surviving plants was under 50.

In another experiment, the effect of position of the inoculum with reference to the plant was studied. For one half of the plants the inoculum was placed at a level 1/2 inch below that of the seed, and for the other half it was placed on the soil surface 1 inch above the seed. Germinated seed bearing sprouts 1 inch long, selected for health and uniformity were used, and the inoculations included both single organisms and several combinations of these.

The inoculum was spaced at 1 inch intervals, this in no case was farther than 1 inch from a plant and was sometimes in contact with the emerging sprouts. There were 20 plants in each set. After 6 weeks growth, when the plants were 18 inches high, the results as to survival and condition were as follows:

Inoculation	Positionof inoculum	Percent of plants surviving	Percent of Normal plants.
HO	below seed	95	50
	above	95	75
RS 341	below	60	55
	above	75	70
* CO (R)	below	70	55
	above	95	75
HO + RS 341	below	70	55
	above	70	55
HO + CO (R)	below	80	60
	above	90	80
none		90	84

* CO (R) is designation for Rice strain of Sclerotium rolfsii.

It is evident that these fungi can attack rice seedlings more effectively when the inoculum is placed close to the seed or roots than when it is on the soil surface near to or even in contact with the sprout. However the resultant effect on growth was so slight up to the stage to which these plants developed, as to preclude material crop damage from this source.

In the previous two experiments the soil was provided with only sufficient moisture to support growth of the plants, which was too dry to permit such growth of the fungi on or just below the soil surface. To obtain information on their infectivity under a more liberal moisture supply, inoculum of these fungi was placed on the soil surface in contact with, or 1/2 inch distant from, sprouts of young rice seedlings immediately after planting, and the soil was kept constantly moistened by a fine water spray for 3 days, after which only ordinary watering was given. 10 seedlings were used in each test. The results 2 weeks following the inoculation were:

Inoculation	Distance of inoculum, inches	Percent of plants surviving	Percent of Normal Plants.
HO- Strain W	0	60	20
	1/2	100	100
HO - W +PO	0	30	10
	1/2	90	80
PO + RS Strain D	0	100	80
	1/2	100	50
HO - W + RS - D	0	50	40
	1/2	100	90
HO - Strain R	0	100	100
+ RS - D	1/2	100	100
HO - R + PO	0	100	90
	1/2	100	100
none		100	100

This experiment showed that some strains of HO could cause a high seedling mortality when the inoculum was in contact with the sprout but little or none when it was separated by 1/2 inch, even under apparently optimum conditions for spread through the soil; other strains were practically non-pathogenic under both conditions. Some evidence also appeared that where HO inoculum was combined with a fungus capable of rapid growth on soil, as certain strains of Rhizoctonia solani, the ability to infect rice was increased.

In all these experiments the plants developed to only an early stage of growth owing to the limited amount of soil in the pots or flats. Several experiments to determine the effect of infection that occurred during the seedling or juvenile stages on ultimate growth and yield records were attempted, but owing to the delayed heading of some varieties in the lengthening days of spring, satisfactory yield records were not obtainable. Seed was sown in a greenhouse bench containing soil enough to support the plants to maturity. Inoculati n was carried out in some instances by adding seed cultures to the soil enough to support the plants to maturity. Inoculation was carried out in some instances by adding seed cultures to the soil at the rate of 100 particles per square foot several days before setting seedlings therein, and in other instances by placing inoculum at measured intervals from the seed at the time of transplanting or shortly thereafter. Portions of the bed were heavi y irrigated in furrows, receiving approximately twice as much water as others. No consistent differences were obtained in weight of tops, number of stalks, or yield of grain between inoculated and check rows, or between plots receiving ordinary vs. heavy watering. The detailed record of one of these experiments is given in the following table, and the general character of plant growth is shown in the accompanying Figs. 1 and 2. The absence of correlation between the total weight of tops and the total number of plants, or even the number of normal plants, surviving in each group emphasizes how largely a reduction in stand of rice plants is compensated by the extra tillering and growth of the survivors.

Inoculation	Per cent of plants surviving		Per cent of normal plants		Average no. of stems per plants		Green weight of tops-gms.	
	var. 1 Ontario.	var. 2 Acadia	var. 1 Ontario.	var. 2 Acadia	var. 1 Ontario.	var. 2 Acadia	var. 1 Ontario.	var. 2 Acadia
HO- N strain								
At time of setting	90	70	90	70	10.2	9.8	745	395
Sprouts 2 inches	70	80	20	60	4.8	9.0	200	270
Sprouts 6 inches	90	90	60	90	5.0	7.3	325	360
HO - N + RS 341								
At time of setting	90	70	80	70	6.8	7.4	505	260
Sprouts 2 inches	80	100	70	90	6.3	7.4	360	410
Sprouts 6 inches	100	100	60	90	4.2	6.4	310	285
RS 341								
At time of setting	90	80	90	80	7.2	7.5	565	300
Sprouts 2 inches	80	100	70	100	5.9	6.5	375	395
Sprouts 6 inches	90	100	70	100	5.2	6.3	365	345
RS 341 + CO (R)								
At time of setting	100	100	100	90	6.1	6.3	415	330
Sprouts 2 inches	100	100	60	70	4.3	5.7	350	280
Sprouts 6 inches	100	100	80	80	4.6	5.7	410	250
CO (R)								
At time of setting	100	90	60	80	4.8	7.0	325	410
Sprouts 2 inches	100	100	70	80	4.3	5.8	350	300
Sprouts 6 inches	100	80	60	60	3.7	6.3	365	260
CO (R) + HO - N								
At time of setting	90	70	80	60	5.0	7.7	365	260
Sprouts 2 inches	100	80	70	70	4.4	7.0	395	260
Sprouts 6 inches	90	100	80	100	5.5	5.6	455	220
CK*								
1	80	100	50	100	4.6	5.4	425	200
2	100	90	30	70	3.3	5.2	240	170
3	100	100	60	60	4.7	3.6	345	120

*Growth in this bed was reduced by washing of soil from roots of plants in watering.

b. Effect of soil temperature and moisture content upon fungus infection of seedlings.

Particular attention was given to determinating how soil temperature and moisture content, including the effect of submergence, may affect the ability of the fungi under test to attack rice seedlings destructively. Two experiments making use of soil temperature control equipment were carried out.

In the first of these the soil was maintained at the following temperature levels 14°, 17°, 20°, 23°, and 26° C, and the moisture content was kept at 15 and 25 per cent in a soil whose maximum moisture-holding capacity was about 55 per cent. This moisture level was considerably lower than the optimum for rice, and was intended to simulate drought conditions. The inoculum consisted of seed cultures of the several fungi tested, both singly and in combination. It was placed in the furrow in which sprouted seed was set at 3 uniform distances, 0, 1/2 and 1 inch, from the seed. The seed was covered with 1 inch of soil and 1 inch of insulating material in order to promote uniformity of moisture and temperature. Ten plants were used for each inoculation unit; they were grown for 30 days and reached a height ranging from 6 inches at the cooler temperature to 15 inches at the warmer. Although pronounced differences in growth were manifest between plants grown at different temperature levels, there were scarcely any significant differences due to the inoculation or the manner in which it was applied. At the lower temperature levels (14 to 20 °) Rhizoctonia solani, either alone or in combination with Sclerotium rolfsii, caused some mortality, but surviving plants compensated for this loss by superior growth so that the ultimate damage was negligible. The details regarding stand and condition of the plants are given below. In this experiment the order of weights (both green and dry) of tops in relation to soil temperature was, in descending order: 20°, 23°, 26°, 17°, 14°.

Inoculation	Position of Inoculation	14° C % stand	14° C % normal	17° % stand	17° % normal	20° % stand	20° % normal	23° % stand	23° % normal	% stand	% normal
RO (R strain *)	0"(next seed)	100	100	100	100	100	90	100	100	100	100
	1/2 inch	100	100	100	100	100	100	100	100	100	100
	1 "	100	100	100	100	100	100	100	100	100	100
RF (P strain)	0"	100	60	100	100	70	60	100	100	100	60
	1/2"	80	70	100	100	80	80	100	100	100	70
	1"	60	50	90	90	70	60	100	100	100	100
PO	0"	100	100	100	100	90	90	100	90	100	100
	1/2"	100	100	100	100	100	100	100	100	90	90
	1"	100	100	100	100	100	100	100	100	100	100
RO (rice strain)	0"	90	80	100	90	100	100	100	100	100	90
	1/2"	100	90	100	100	90	80	100	100	100	100
	1"	100	80	100	100	100	100	100	100	100	100
RS + CO (R)	0"	100	100	60	60	100	100	100	80	100	100
	1/2"	100	100	90	90	100	90	100	80	100	100
	1"	100	100	100	100	100	100	100	90	100	100

Check

1	100	100	100	100	100	100	100	90	100	100
2	100	100	100	100	100	100	100	90	100	100
3	100	100	100	100	100	100	100	100	100	100

* This strain was later found to be weakly pathogenic in comparison with other HC strains.

In the second experiment on the relation of soil temperature to fungus infection of rice seedlings, the temperature levels were 17, 20, 23, 26 and 30° C. The soil moisture content ranged between 20 and 30 per cent, but was predominantly 22 to 26 per cent, i. e., slightly less than half saturated. At this average level, however, the surface soil, including the horizon in which the inoculum was placed, was saturated for a while during the periodic waterings, 3 to 4 times each week. This moisture content appeared to be about optimum for rice under conditions simulating upland culture. After 7 weeks of growth under these conditions the vessels holding the plants were moved outdoors where they were all exposed to similar temperature conditions and the soil was kept flooded. It would have been desirable to hold them until seed ripened, but heading was delayed (probably on account of the long days) and no important differences in growth or condition of the plants appeared; accordingly they were cut after 7 weeks. The inoculum consisted of seed cultures and was placed on the soil surface, either next the sprout of a young seedling or at a distance of 1 inch from each plant, and was lightly covered with shredded sphagnum moss for insulation. Ten plants constituted an inoculation unit. Figs. 3 to 6 show the type of culture vessel and manner of growth of representative plants at the conclusion of the period of constant temperature and moisture supply. The final results on stand and yield are given in the following table. The average values for stand and yield in the different temperature and inoculation groups are inserted to facilitate quick comparison, but are without statistical significance in view of the variability shown between comparable units, e. g. the two check rows in the same culture vessel.

No consistent or important effects resulted from inoculation with any of these fungi under these conditions, though in a few instances there was an appreciable seedling mortality. This experiment emphasized again the compensatory effect of increased growth in surviving plants when additional space was afforded them through stand reduction by seedling blights, e. g. in one instance a stand of 10 seedlings was reduced to 5 apparently by Helminthosporium infection, but the surviving plants produced a top growth equal to the average yield where no reduction of stand occurred.

Inoculation	Position of Inoculation	% stand	green wt. gms	% stand	green wt. gms	% stand	green wt. gms	% stand	green wt. gms	% stand	green wt. gms
HO V strain	next sprout	90	135	100	220	100	200	100	145	90	255
	1"	90	195	100	205	100	150	90	265	90	155
HO+RS	next sprout	100	260	90	145	100	180	100	225	80	180
	1"	90	140	100	240	100	180	90	210	90	210
HO+CO	next sprout	100	170	100	140	80	130	80	130	90	295
	1"	90	145	90	200	100	250	90	265	100	240
HO+CO	next sprout	100	170	90	175	100	200	90	160	90	220
	1"	80	170	90	180	100	170	90	240	90	230
HO+RS+CO	next sprout	70	125	100	180	100	200	50	200	90	270
	1"	90	195	100	210	100	165	80	175	90	215
Check	1	100	205	100	215	100	245	100	220	90	245
	2	100	140	100	170	100	155	100	215	100	210

Summary of preceding table showing group averages.

a. Results with different inoculation--all temperatures.

	Average stand %	Average weight of tops gm
HO-W next sprout	96	191
1 inch	95	194
HO + RS next sprout	95	198
1 inch	95	196
HO + CO next sprout	90	173
1 inch	95	220
RS + CO next sprout	95	185
1 inch	90	198
HO + RS next sprout	82	195
+ CO 1 inch	92	192
Check 1	98	226
" 2	100	178

b. Results with different temperatures--all inoculations.

17° C	92	171
20	97	190
23	98	185
26	88	204
30	90	227

C. Effect of submergence upon growth of seedling blight fungi

The experiments heretofore reported were made on rice plants growing in soil ranging from relatively dry to moist but not saturated or submerged, thus simulating the conditions under which rice seedlings normally grow. Information was also desired on the growth and infectivity of the seedling blight fungi when the inoculum was either submerged or floating on water.

Preliminary tests, in which rice plants at different stages of growth and growing in flooded soil were inoculated with seed cultures of these fungi, showed that they resisted infection fully as successfully as when growing in dry or moist soil. The seed inoculum tended to remain afloat for indefinite periods and even made considerable growth under these conditions, especially CO which often formed an extensive mycelial mat bearing secondary sclerotia. It was intolerant of submergence however, whereas HO and RS survived and grew for some time under water. Accordingly, a more detailed study was made of the ability of these fungi to grow under various conditions of submergence.

For this purpose cylindrical bottles of 6 ounce capacity were filled with water to different levels from 1 to 3 inches. For testing growth when submerged, inoculum (usually 1 sorghum seed or pellet) was wedged into a piece of glass tubing 1 cm long and dropped into the bottle, where it remained at the bottom. For testing its ability to grow on the surface, it usually sufficed to place dry seed inoculum on the water surface, where it remained afloat, or pellet inoculum was floated on thin discs of cork. The ability of these fungi to grow (from seed inoculum) at various levels under water is shown in the following table, the amount of growth being indicated by the symbols O, ±, +, and ++ in increasing order.

Organism	Surface	Submerged 1 inch	2 inches	3 inches
HO	±	++	++	++
PO	±	+	+	+
RS	++	++	+	+
CO	++	±	±	±

Figs. 7 to 10 show the growth characteristics of several of these fungi under these conditions.

Next rice seedlings were grown in similar bottles, using an identical amount of soil in each, covered to the same level with water, and selecting the seedlings carefully for uniformity of size and condition. The small growing space afforded by these bottles confined the inoculum to more or less close contact with the seedlings. Five plants were used for each kind of inoculation and the cultural conditions were kept as uniform as possible so that the individual plants in each unit were similar to a high degree.

The first inoculation was made when the shoots were 6 to 8 inches high, and was repeated 2 weeks later when they had grown to 12 inches and were 4 weeks old. They were kept under observation for 3 weeks following the inoculation but a large majority of them remained to all appearance normal. There was no mortality from any kind or combination of inoculation, and the only pathological indications were a generally superficial leaf-sheath rot that only occasionally entered the culm, and slight necrosis of the upper roots. These manifestations resulted chiefly from floating inoculum, and especially from CO or combinations including this fungus. In a few instances CO produced sclerotia that could be found attached to necrotic leaf sheaths or floating on the water, and they ultimately sank. Tisdale (Jour. Agric. Res. 21, p. 654) found that sclerotia of this fungus might remain viable under field conditions during the period of irrigation, and resume development on the roots and stems when the fields were drained.

The results of an experiment to test the effect of submergence on this method of inoculation are given below. Unless otherwise stated the inoculum consisted of seed cultures of the respective fungi.

	Submerged		Floating	
Inoculum	Pct. normal	Pct. Sheath rot	Pct. normal	Pct. sheath rot.
HO - 1 (M type)	80	20	100	0
HO - 7 (MC type)	100	0	80	20
PO	100	0	100	0
RS 341	60	40	100	0
CO (R)	100	0	100	0
HO - 1 + PO	100	0	100	0
HO - 1 + RS 341	100	0	100	0
HO - 1 + CO (R)	100	0	40	60
PO + RS 341	100		60	40
PO + CO (R)	100	0	60	40
RS 341 + CO (R)	100	0	60	40
RS 341 - pellet	100	0	100	0
CO (R) - pellet	80	20*	60	40
RS 341 + CO(R) - pellet	60	40*	80	20
Check	100	0	100	0

* The pellets disintegrated and some of the material floated.

A number of plants thus inoculated and superficially infected were grown on until heads formed, and their production of straw and grain compared with control plants. Significant differences in yield were not observed, the production of infected plants equalling or exceeding that of control plants about as often as it fell below. Only a few instances of a fatal stem rot or shoot blight occurred, and these probably resulted from complications due to natural infection of the seed with HO.

Some of the plants that developed sheath rot in their early stage of growth, but matured normally, were cut back and reinoculated. A large majority of them sprouted normally even after this treatment, which was intended to afford an optimum chance for latent infections of the root collar to become evident or for new infections of the cut stems to spread into young sprouts.

Photographs illustrating the set-up used for inoculating plants grown in flooded soil, and showing examples of the negative effects of such inoculation, are given in Figs. 11 to 14.

(2) Combination inoculations with two or more pathogens to determine their compatibility and their capacity to produce disease complexes of increased severity.

Some of the results of inoculating plants with combinations of pathogenic fungi have been stated in Section 1 above, but a more detailed study of the ability of certain combinations of inoculum to grow together and to infect plants also was made.

Compatibility of growth was tested by double inoculation, using seed cultures as inoculum, (1) on moist blotting paper in petri dishes, and (2) in bottles of water under several levels of submergence; growth was estimated visually. There was no evidence of antagonism between any combinations of HO, RS, and CO in the several strains tested, although RS and CO predominated over HO under aerobic conditions, and RS and HO over CO under reduced aeration; RS and CO grew well together on the surface and RS and HO grew normally when submerged. Examples are shown in Figs. and .

Rice plants growing in ordinary and also flooded soil were inoculated at various stages of development with these fungi, singly and in pairs. Inoculations were carried out in various ways as previously described and as summarized below:

(1) On soil surface, seeds or pellets, soil not flooded.
(2) As in 1 but soil covered with 1 inch of water.
(3) On water surface 1 inch above soil.
(4) Macerated fresh inoculum, or shredded dry inoculum on foliage and on soil surface.

The indications of pathogenicity were not marked in any instance, therefore the details of these experiments need not be reviewed. However, in certain instances infectionof vital tissues occurred in addition to the more or less saprophytic basal sheath rot, and this was manifest in discoloration and necrosis in the inner tissues of the culm, and especially of the basal node or root collar. Such infections were in general limited to plants not over 3 to 4 weeks old and 6 to 12 inches tall when inoculated; plants that escaped infection at this stage did not become infected subsequently through proximity to diseased plants. It was necessary to maintain approximately optimum conditions for these fungi-- a temperature range 22 to 30° C (72 to 85° F) and a saturated atmosphere for a minimum of 2 to 3 days-- in order to induce infection to this extent.

An example of the results of one inoculation experiment in which the plants were grown in pots of nearly saturated soil, and incubated either in a closed dark chamber or in a greenhouse inclosure maintained at nearly 100 per cent relative humidity, is shown in the following table, and is illustrated in Figs. 15 to 34. There were 3 pots (3 inch) each containing 5 plants of Caloro variety, 30 days old, in each inoculation unit.

Inoculation	Incubation	Results
RS341 sorghum	30° C, 3 days	Superficial basal sheath rot only
"	26° , 4 days	"
"	22° , 5 days	Normal growth
"	Greenhouse	" "
RS 341 pellets	30° , 3 days	Normal growth
"	26° , 4 days	" "
"	22° , 5 days	" "
"	Greenhouse	
CO(R) sorghum seed	30° , 3 days	Basal sheath rot also involving stems; plants stunted.
"	26° , 4 days	As above, but less advanced.
"	22° , 5 days	Slight and mostly superficial sheath rot.
"	Greenhouse	Superficial sheath rot, growth not affected.
CO(R) pellets	30° , 3 days	Normal growth.
"	26° , 4 days	" "
"	22° , 5 days	" "
"	Greenhouse	" "
RS 341 + CO (R) Sorghum	30° , 3 days	Basal sheath rot and stem streak or necrosis
"	26° , 4 days	"
"	22° , 5 days	Superficial sheath rot.
"	Greenhouse	Normal growth
RS 341 + CO(R) pellets	30° , 3 days	Normal growth
"	26° , 4 days	" "
"	22° , 5 days	" "
	Greenhouse	" "

HO (T strain) sorghum	30°, 3 days	Superficial basal sheath streak
"	26°, 4 days	"
"	22°, 5 days	"
"	Greenhouse	Normal growth
HO - T + CO (R) sorghum	30°, 3 days	Severe sheath rot and stem streak, plants stunted
"	26°, 4 days	"
"	22°, 5 days	"
"	Greenhouse	Superficial sheath rot.

In general, the sheath rot and stem streak induced by HO + CO had the appearance of greater injury, and occurred through a wider range of incubating conditions, than any other effect of these inoculations. Plants that were thus infected would probably yield poorly or even be eliminated under ordinary field conditions.

Some examples of the results of similar inoculation experiments using combinations of fungi in which the plants grew in submerged soil are shown in Figs. 11 to 14, but in view of the absence of marked pathological effects resulting from any form of inoculation, even when repeated twice on the same plants, the details are omitted.

(3) Inoculation technique with (a) seedlings and (b) plants growing with submerged roots.

Most of the details of these studies have been reported under preceding headings, as on pp. 16 - 18.

A few general conclusions based on observation rather than completely verified experiments are tentatively advanced:

a. Rice seedlings are most readily infected with HO, RS and CO by means of soil inoculation prior to sowing seed or transplanting sprouted seed. At least with seed inoculum of the type chiefly used, the inoculum must be within about 1 inch of the seed or sprout to cause infection before the plant grows out of its period of susceptibility.

b. No method was found of infecting the submerged parts of rice plants that were growing in flooded soil with any of these fungi.

c. The simplest and most effective method of inoculating rice leaves with HO, so as to produce uniform leaf spot infections, was to wipe each leaf between one's fingers dipped each time in a heavy suspension of conidia. The plants were then placed in an inoculating case in which the atmosphere was periodically saturated with fog from a humidifying machine, usually requiring operation for about 5 minutes each half hour for 12 to 16 hours. Lesions were evident in 36 to 40 hours, and a complete record could be made on the 4th day. Plants having up to 3 developed leaves were most suitable for such inoculation; the leaves of plants approaching full size were much more difficult to infect. See Figs. 38 - 41.

(4) Measurement of plant injury resulting from infection

No satisfactory means of expressing quantitatively the results of infection of rice by these fungi was found; however the subject was very superficially studied. Reduction of stand by seedling blight tended to be compensated by the superior growth of surviving healthy plants. Some of the inoculations caused a leaf sheath rot that resulted in visible stunting at least for a while, but such infections were superficial and the plants tended to outgrow them unless the inoculation was repeated. In no instance was a fatal stem rot or shoot blight produced, and under greenhouse conditions no statistically significant differences in green weight or yield of grain resulted from such sheath infections.

The leaf spot infections induced by artificial inoculaton of juvenile plants with HO did not result in measurable damage. Even heavily spotted leaves remained green about as long as normal leaves of the same stage of growth. There was only a slow spread of infection, and usually little or none, from infected leaves to those subsequently developing, even when the plants were alternately subjected to a dry atmosphere (to induce HO sporulation) and then to a fog-saturated atmosphere with an air movement of about 10 miles per hour which was maintained for several hours up to 2 days.

The most injurious effect of HO infection appeared to be that originating in the seedling from infected seed. This not only caused marked stunting of the seedling in most cases, but even where non-fatal such infection was not outgrown, both leaf lesions and especially stem lesions, in a manner suggestive of systemic infection, continuing to develop during the life of the plant, and HO sporulated on these lesions.

It would appear worth while to investigate further the possibilities of disseminating HO by means of infected seed, both of rice and of other suscepts such as crabgrass, pigeon grass, etc.

(5) Comparative infectivity of different strains of Helminthosporium oryzae in relation to conidial and mycelial growth habit.

No attempt was made to assemble isolates of HO from a number of sources differing in locality, variety of host, and type of lesion from which they were obtained. Our first culture of HO was obtained from Dr. H. A. Rodenhiser, and had been isolated from and previously used in pathogenicity tests on rice. When received it characteristically produced only mycelium, or a few depauperate conidia on artificial culture media. It was difficult to obtain infection of rice by artificial inoculation of foliage, although this could be accomplished to some extent by making a suspension of mycelial fragments in a Waring blender.

Since rice seed of several varieties was at hand which bore HO lesions on the seed coat, or gave rise to HO-infected seedlings, a number of new cultures of HO were obtained from these sources. These fresh isolates were much more vigorous in growth than our first culture, and they produced conidia readily on practically all artificial media that were tried. They were initially in the growth state termed intermediate or mixed (C type) ie. they produced both conidia and mycelium, and also gave rise to different types of growth appearing as irregular tufts and V-shaped segments when grown in petri dish cultures. From these "intermediate" cultures, pure conidial (C) strains could be isolated readily by the dilution plate method. These conidial strains were stable, or practically so, as long as they were maintained by frequent transfers of conidia only, but they tended to throw off mycelial segregates which soon dominated the culture, especially when grown in test-tubes. Successive transfers of mycelium soon resulted in the segregation of pure mycelial (M) types which refused to sporulate on any media, or by any manipulation, that was tested. These culture types are an expression of the "dual phenomenon", which was first identified by H. N. Hansen (Mycologia 20: 442-455, 1935) as characteristic of many of the Fungi Imperfecti, and which is doubtless an important factor in the reported frequency of mutation or saltation in Helminthosporium. The characteristic appearance of these 3 types of cultures of HO in petri dishes is shown in Figs. 35, 36 and 37.

According to the experience acquired in the present work, the C type of culture produces infection much more readily when inoculated on rice leaves than do the MC or M. types. It is often difficult to obtain any infection at all with the pure M type. However, since the M and MC types produce mycelium in much larger amounts than the C type, it was thought that perhaps these types might more readily induce seedling blight, or stem and basal sheath lesions, when the inoculum was placed on the soil than the C type. Tests were made comparing the 3 types as produced on sorghum seed and placing the inoculum at different distances from rice seedlings grown singly in pots. The type and extent of growth were compared also on an inert substrate such as moist blotting paper in petri dishes, on soil, and on the surface of and also submerged in water.

Although fully conclusive results were not obtained, it is fairly certain that the M and the MC culture types are not able to infect rice seedlings more readily than the C type, or do they spread more rapidly over soil or other substrates. The mycelial mats produced by C types on the surface of paper soil are usually conspicuously thinner than those of M and MC types, but they cover about the same area in the same time. The amount of mycelium produced when submerged in water is essentially the same in the 3 types. Old, "degenerate" cultures of the pure M type may show less growth than MC types on an inert substrate, and are definitely less aggressive in attacking plants under all the conditions tested than are the C and MC types.

It is therefore essential that cultures be kept in the C, or at least the MC, condition by frequent transfers (perhaps once a month will suffice) using a conidial suspension in water to inoculate the fresh medium, or they should be periodically "rejuvenated" by inoculating plants and reisolating therefrom. The superficial growth of the C type on sorghum seed appears as a fine greenish-black felt, and consists almost exclusively of conidia. Produced in this way and stored dry on the seeds, the conidia remained viable and infective for at least 3 months.

Although any HO culture appears to be more infective in the C and MC state, a satisfactory answer was not obtained to the question whether races or biotypes of this species exist in nature which differ significantly in pathogenicity. Some 20 HO isolates from different sources were tested for this purpose on 3 varieties of rice, but these isolates differed among themselves as to growth type, and could not be compared on the basis of conidial suspensions of uniform concentration. All that was learned from these tests was that the severity of infection when expressed as total number of infections, or number of infections per leaf, was more dependent on the concentration of conidia in the inoculus than upon the source of the culture, thus the C types consistently produced denser infection than MC types, and M types failed almost entirely. One of our C type isolates from a naturally infected seedling produced conspicuously more numerous lesions in 2 separate tests on 3 varieties of rice than the C type obtained from Dr. E. C. Tullis last fall, but this result may be due to the difference in length of time that the two cultures were kept on artificial media.

A comparison was made of several methods of inoculating rice plants with HO. The methods were:

(1) Conidial or mycelial suspension sprayed on leaves with a hand atomizer.

(2) Dry conidia from sorghum seed or bran cultures applied by (a) dusting over leaves previously sprayed with water, or (b) wiped over dry leaves which were then sprayed with water or were coated with "dew" by placing in the humidifying chamber.

(3) Wiping leaves between one's fingers after they were dipped in a dense conidial suspension.

After applying the inoculum the plants were kept overnight (16 to 18 hours) in a humidifying chamber, during which time the leaves were constantly covered with fine dew. The plants were then allowed to dry off, but were still kept under partial shade. HO lesions were visible in 30 to 40 hours, and a complete record of infection could be made in 4 to 5 days. Longer periods of incubation in an atmosphere saturated with fog were tried but with no benefit.

In general, Method 3 produced the greatest numbers of and the most uniform lesions. It was also more rapid than Method 1 when a hand atomizer of the household type was used. Method 2 gave erratic results by both variations, occasionally producing large and numerous lesions, but often few or none. Figs. 38 to 41 show examples of the results.

The most effective conditions for infection were obtained by operating the humidifying machine only at close enough intervals, and for sufficient lengths of time, to preserve a thin coating of dew on the foliage. This appeared to require about 5 minutes of operation every half hour. Constant operation for the whole period of incubation reduced the number of infections, probably due to dripping of inoculum from the leaves. The humidostat supplied by the manufacturer with our machine could not be adjusted to operate automatically within the range 90 to 100 per cent relative humidity. It either tended to remain "frozen" in the "on" position in a fully saturated atmosphere, which resulted in excessive moisture deposition and drip, or it failed to come on as the humidity dropped to 90 in time to prevent temporary drying of the leaves.

The rice varieties Caloro, Acadia and Nataribune were about equally susceptible to the strains of HO that were tested. The lesions were somewhat larger and tended to coalesce in Nataribune than in the other varieties. Our Chinese variety of unknown identity was somewhat resistant, as the lesions were somewhat fewer and much reduced in size.

In addition to rice the following plants were infected by artificial inoculation, giving rise to typical "eye spot" lesions on the foliage: Digitaria sanguinalis (crabgrass), Setaria viridis (green bristlegrass), and Cyperus esculentus (tuberous sedge).

(6) Comparative infectivity of different strains of Rhizoctonia spp. for rice.

A brief study of this subject was made because of: (1) the existence of a number of reports in literature of diseases of rice attributed to Rhizoctonia spp. and particularly to R. solani; these diseases include stem and root rots of seedlings, and a leaf sheath disease ("sheath spot") of adult plants (T.C. Ryker and F.S. Gooch--Rhizoctonia sheath spot of rice. Phytopathology 28: 238-246, 1938); (2) the indicated compatibility between Rhizoctonia and CO and the increased virulence of dual infections on certain plants.

The Rhizoctonia sp. described as R. oryzae by Ryker and Gooch was not obtained for this study, but a number of variant strains of R. solani were assembled. These included strains from a wide geographic range in the U. S., and from a number of different host plants--cereals, cotton, sugar beet, potato, tobacco, and miscellaneous. Several strains were associated with leaf spots and other diseases of aerial parts of their respective hosts in the field. In view of the present inclusive concept of the fungi referred to the species R. solani (=Pellicularia filamentosa (Pat.) (Rogers) and the fact that many forms thus identified are pathogenic to foliage, it is probable that R. oryzae Ryker & Gooch also belongs here. It is probable also that the strains tested in this study are fairly representative of the effects of this composite species on rice, although other strains showing minor differences in pathogenicity may exist. Among the 21 cultures tested, for ability to attack rice seedlings, both before and after emergence, and also to infect aerial parts of older plants, strain 341, isolated from cotton in North Carolina proved as infective as any, and more so than most strains; accordingly it was used in all combination inoculations with CO, HO, and PO. When inoculated alone it caused only minor infections of the roots, root collar, and basal leaf sheaths. Accordingly, fungi of this species are considered to have but very little promise for the present purpose.

Strain 341 exhibited a high level of virulence for several other plants including barley, beans, soybeans, and tomatoes. Either alone, or in combination with CO, it offers definite possibilities of damage to these and other crop plants.

All of the tested Rhizoctonia strains grew readily on seed media of various kinds, and these preparations could be easily separated into particles and disseminated; they were also viable after prolonged storage and desiccation.

(7) Comparative growth and infectivity of natural seed vs. pellet inoculum.

A comparison was made of the growth from two types of inoculum, sorghum seed and artificial pellets furnished by Dr. A. G. Norman, including cultures of RS 341 (a strain of Rhizoctonia solani), CO (rice strain), and the two organisms together. On a substrate such as non-sterile, moist blotting paper, there was little to choose between the two types of inoculum. RS grew somewhat more rapidly from pellets than from seed, especially at a moderate temperature (18° C), but there was less difference at 28°. Both covered a 4 inch circle within 3 and 6 days respectively at the two temperatures. (Figs. and) CO sometimes grew well from pellets but was often (in about 50 per cent of the examples) completely inhibited by contaminants, chiefly Aspergillus, Penicillium and Rhizopus. CO grew vigorously from sorghum seed, producing practically pure cultures, covering a 4 inch circle in less than 3 days at 28°. When the two organisms were inoculated together, RS outgrew CO and restricted its development to the center of the mat, or the proximity of the inoculum.

On a water surface, or when submerged in water, the advantage in growth was greatly in favor of the seed inoculum. The only consistent growth from pellet inoculum was that of RS, from either RS or RS + CO pellets, when the inoculum remained afloat. When submerged from 1 to 3 inches, bacterial contamination restricted or completely suppressed the growth of both organisms. With seed inoculum, growth of both RS and CO, separately or together, was vigorous on the surface, and RS grew nearly as well at the tested depths of submergence, though CO grew weakly when submerged at all. (Figs. 7 to 10).

When used to inoculate plants, the results with pellet inoculum of CO were disappointing, although with RS the pellet inoculum was about as effective as the seed. Figs. 42 to 48 show the results of inoculating bean plants with the two kinds of inoculum. Whereas CO from sorghum seed inoculum generally caused about 50 per cent infection and mortality, no CO infection from pellets was obtained in numerous trials, and for the most part there was neither mycelial growth nor sclerotial production by this organism, owing to heavy contamination with molds. These pellets were perhaps too old so that CO had weakened or died out, but they failed to cause CO infection of a susceptible plant, such as beans, also when first tested and not over 2 weeks old. Combination inoculations with CO and various strains of RS usually resulted in over 50 per cent and often total infection. An insufficient number of tests were made to show significant differences in infection rate between RS strains alone as compared with CO combinations, but the plant mortality appeared greater in the dual inoculations. On beans, RS alone produces numerous, and ultimately destructive but not immediately fatal, stem cankers, whereas in combination with CO a fatal stem rot usually results.

The potentialities of a combination inoculum of RS and CO against beans seem worthy of further investigation in view of the importance of both common beans and soybeans in the Japanese diet, and of the latter also as a source of animal feed and probably of munitions.

In respect to infectivity for rice, the pellet inoculum was generally unsatisfactory. This naturally follows from the facts that the pellet inoculum used in these tests was not an effective source of CO infection, and that no strain of RS that was tried proved virulent for rice regardless of the type of inoculum or manner of inoculation. The negative results from pellet inoculum on rice are shown in Figs. 27 and 29, and the contrast with seed inoculum of CO is shown in Fig. 28.

(8) Comparative infectivity of different strains of Rhizoctonia alone and in combination with Sclerotium rolfsii for beans.

Most of the strains of Rhizoctonia assembled for testing on rice were tested also on common beans, and several of them on soybeans. Marked differences in virulence were observed but no strain was consistently superior to RS 341. The essential results with this strain used alone and in dual inoculations with CO are summarized in the preceding section and are illustrated in Figs. 15 to 34. The detailed results are available in the event of further interest along this line.

(9) Summary and conclusions

The following fungi, Helminthosporium oryzae, Piricularia oryzae, Rhizoctonia solani, and Sclerotium rolfsii proved incapable of seriously damaging rice plants when inoculum of the seed (or in some cases of the pellet) type was dropped on soil containing plants in various growth stages from emergence of sprouts to the size at which rice is normally transplanted (about 1 month old). Significant stand reduction (up to 50 per cent) resulted only when the soil was heavily inoculated before the seed was sown or seedlings transplanted into it.

Because of its free tillering habits rice has a high capacity to compensate for stand reduction in early stages of growth if the surviving plants remain healthy. A 50 per cent stand reduction may not, in some cases, cause an appreciable loss in ultimate growth and yield.

Under very favorable environmental conditions any of these fungi may cause a basal leaf-sheath rot, which sometimes penetrates the culm and the root collar. Appreciable stunting of growth results, which perhaps under field conditions would result in barrenness. The most effective means found for inducing this leaf sheath and stem rot in these experiments was a combination inoculation with CO and HO.

Rice plants growing in submerged soil were less readily infected by artificial inoculation with these organisms than were plants in more or less dry soil. However, HO and RS can grow quite successfully when submerged to at least 3 inches in water whereas CO scarcely tolerates submergence at all.

Significant differences occur in the virulence of different isolates of HO. Strains of the conidial and intermediate types were much more effective in producing typical leaf and stem spot infections than were strains of the mycelial type. Such strains were also fully as effective in causing basal leaf sheath and root collar infections as the mycelial types, although the latter generally grew more vigorously on artificial media and on soil.

Seed inoculum of CO proved more effective than pellet inoculum in these tests in which growth on moist paper, on soil, in water, and on various plants was compared. There was no difference in the growth of seed vs. pellet inoculum of RS, except in water, in which the seed inoculum grew better.

Some strains of RS are highly pathogenic to common beans and soybeans, and when inoculated together with CO may cause severe and often fatal infection of these plants.

Fig. 1. Greenhouse plot of rice (variety Wataribune) artificially inoculated with seedling root- and stem-infecting fungi. Portions are shown of plots inoculated with HO, RS, and CO but growth is essentially uniform throughout.

Fig. 2. Detailed view of portion of Fig. 1. The 3 rows to left of stake were inoculated with HO, the 2 rows to right with HO + RS. Results negative.

Fig. 3
Inoc. with HO + CO

Fig. 4
Check

Fig. 5
Inoc. with HO + RS + CO

Fig. 6
Same as Fig. 5; left row inoc. 1 inch from sprout, right row next sprout.

Figs. 3 to 6. Examples of growth of rice as affected by inoculation of seedlings with root- and stem-infesting fungi. These plants were grown at a constant soil temperature of 20° C. Others were grown at 17, 23, 26 and 30°. Inoculation as indicated under each figure.

Fig. 7. Effect of submergence on growth of inoculum. Double inoculation with RS + CO. Left, submerged 2 inches; right, submerged 1 inch. Only RS grew when submerged.

Fig. [illegible]. Left, inoculum placed on surface; right, submerged 3 inches. Note growth of CO with formation of secondary sclerotia at left.

Fig. 9. Effect of submergence on growth of inoculum. Double inoculation with HD + RS. Left, at depth 2 inches; right, at depth 1 inch. Some growth of both fungi at both depths.

Fig. 10. Similar. Left inoculum on surface; right, submerged 3 inches. These two fungi make some growth at all positions.

Fig. 11. Effect of submerged vs. floating inoculum. Seed culture of CO submerged, results negative.

Fig. 12. Similar; CO inoculum floating. Results negative, although secondary sclerotia were formed and remained floating on water surface.

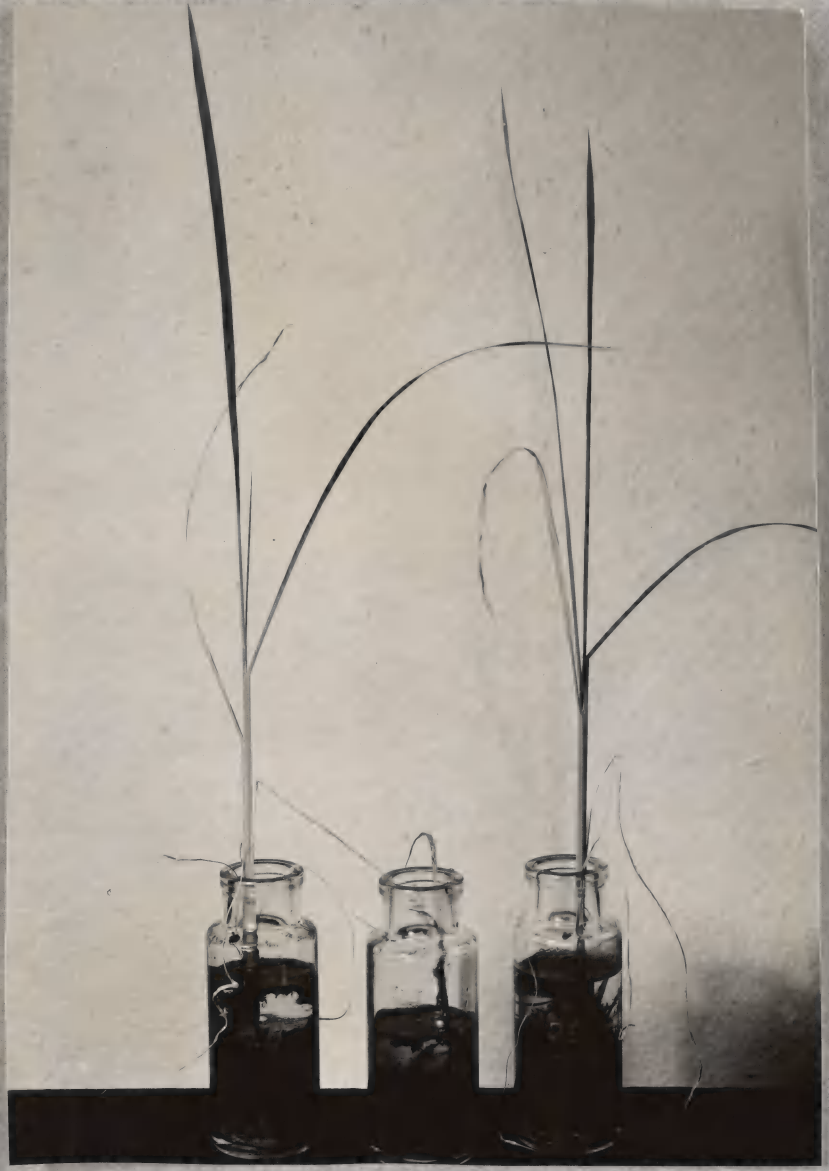

Fig. 13. Effect of submerged vs. floating inoculum. Seed culture of RD placed on water surface. Middle plant infected but possibly natural infection from seed; others negative.

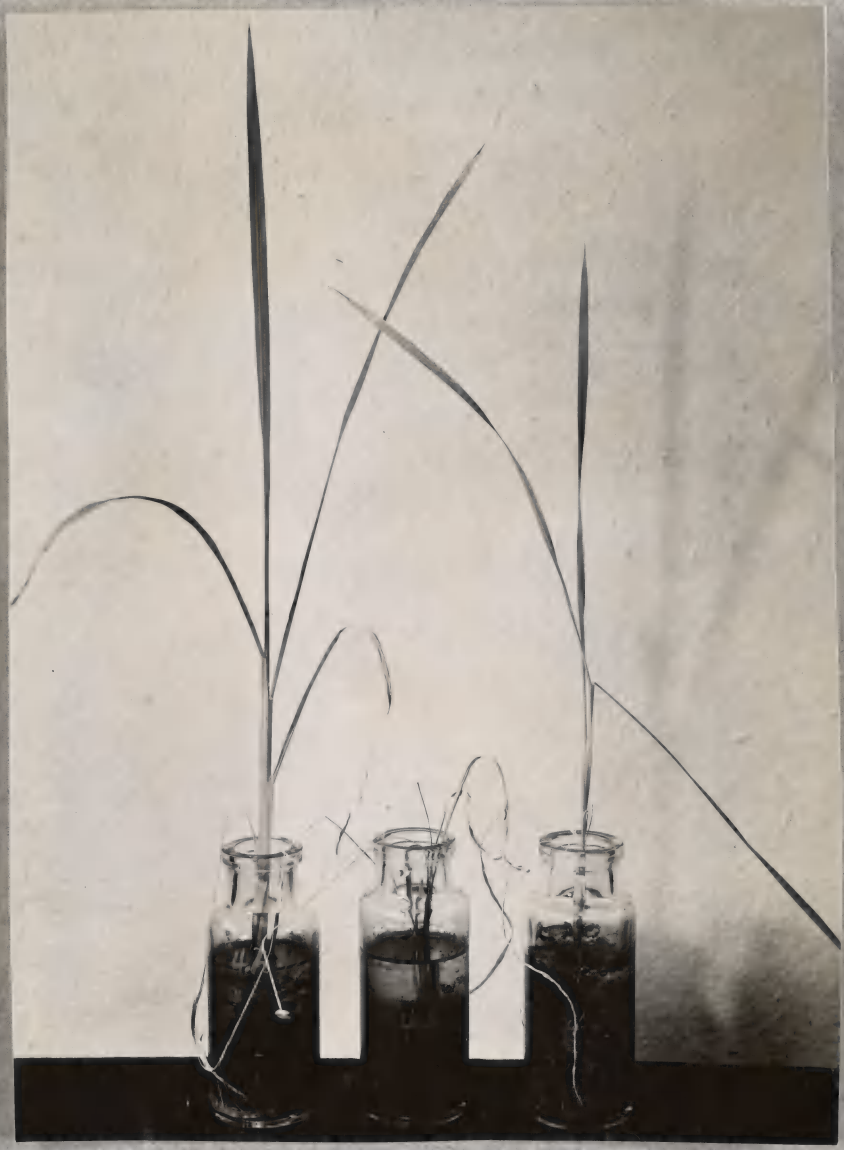

Fig. 14. Similar; RS inoculum floating. Middle plant succumbed to root- and stem rot. All inoculations negative with submerged inoculum.

Fig. 15. CO on sorghum seed.
30° C, 3 days.

Fig. 16. RS 341 on sorghum seed.
30° C, 3 days.

Fig. 17. CO + RS 341 on sorghum.
30° C, 3 days.

Fig. 18. CO + HO on sorghum.
30° C, 3 days.

Figs. 15 to 18. Results of inoculation of rice seedlings with various fungi as evident at conclusion of incubation period at the stated conditions.

Fig. 19. CO on sorghum seed.
26° C, 4 days.

Fig. 20. CO pellet (no growth)
26° C, 4 days.

Fig. 21. CO + RS 341 on sorghum.
26° C, 4 days.

Fig. 22. CO + RO on sorghum.

Figs. 19 to 22. Results of inoculating rice seedlings, cont.

Fig. 23. CO on sorghum.
22° C, 5 days.

Fig. 24. RS 341 on sorghum.
22° , 5 days.

Fig. 25. CO + RS 341
22° , 5 days.

Fig. 26. CO + RO
22° C, 5 days.

Figs. 23 to 26. Results of inoculating rice seedlings, cont.

Fig. 27. CO, RS 341, and CO + RS 341.
On pellets, 30° , 3 days. No visible growth.

Fig. 28. CO on pellet, left; on sorghum seed right. Note mycelium and sclerotia in latter.

Figs. 27 to 29. Results of inoculating rice seedlings, cont.

Fig. 29. CO, RS 341, and CO + RS 341
On pellets, 22° C 5 days. Sl[illegible] discoloration of base of culms due to RS.

Fig. 30. Results of inoculating rice seedlings, cont. From left to right the plants were inoculated with sorghum cultures of CO, CO + RS 341, and CO + RO. No effect of such inoculation on the plants under greenhouse conditions.

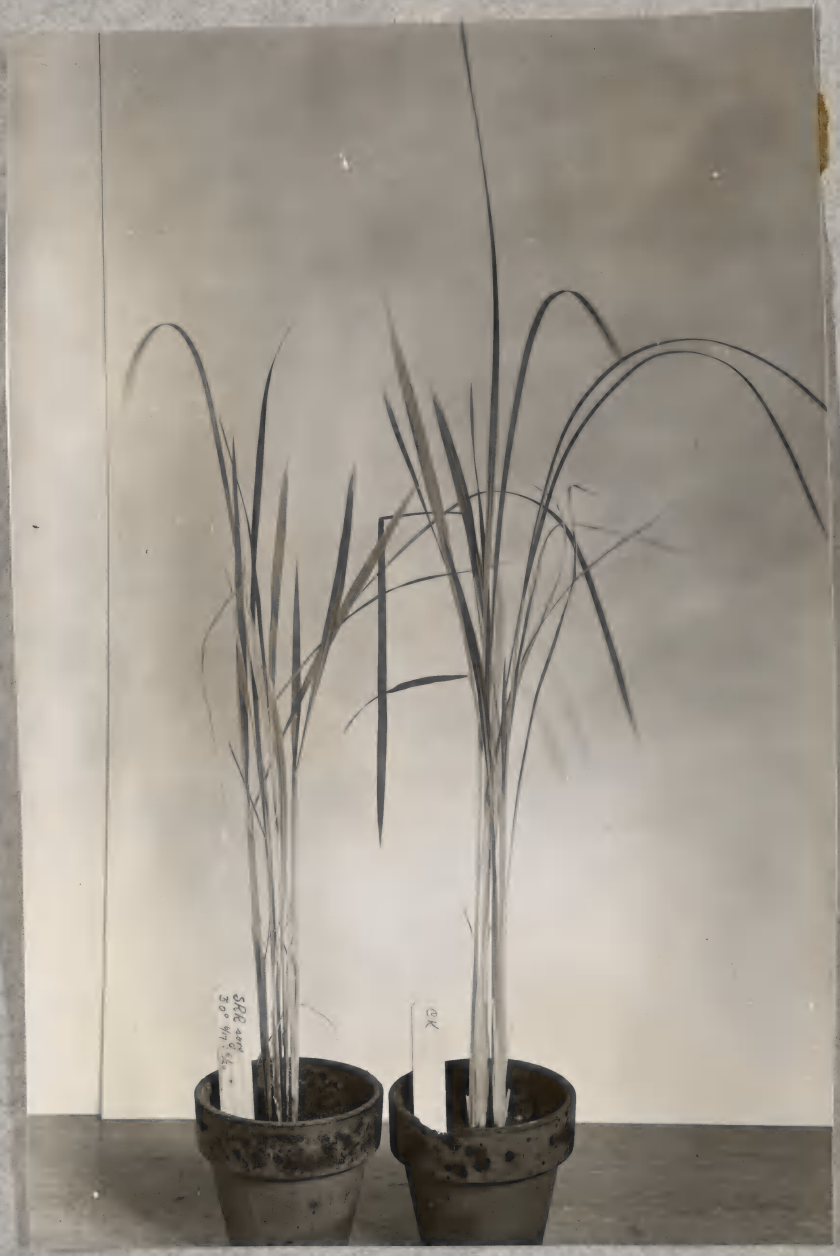

Fig. 31. CO on sorghum left, check right. 30° C, 3 days

Fig. 32. CO + RS 341 on sorghum left, Check right. 26°, 4 days.

Figs. 31 and 32. Results of inoculating rice plants, cont. Condition 2 weeks after inoculation shows incipient sheath rot.

Fig. 33. HO on sorghum left, check right.
30° C, 3 days. Results negative.

Fig. 34. CO + HO sorghum
30° , 3 days. Sheath and stem discoloration.

Figs. 33 and 34. Results of inoculating rice seedlings, cont.
Condition of plants 2 weeks after inoculation.

Fig. 35. Conidial culture type of HD

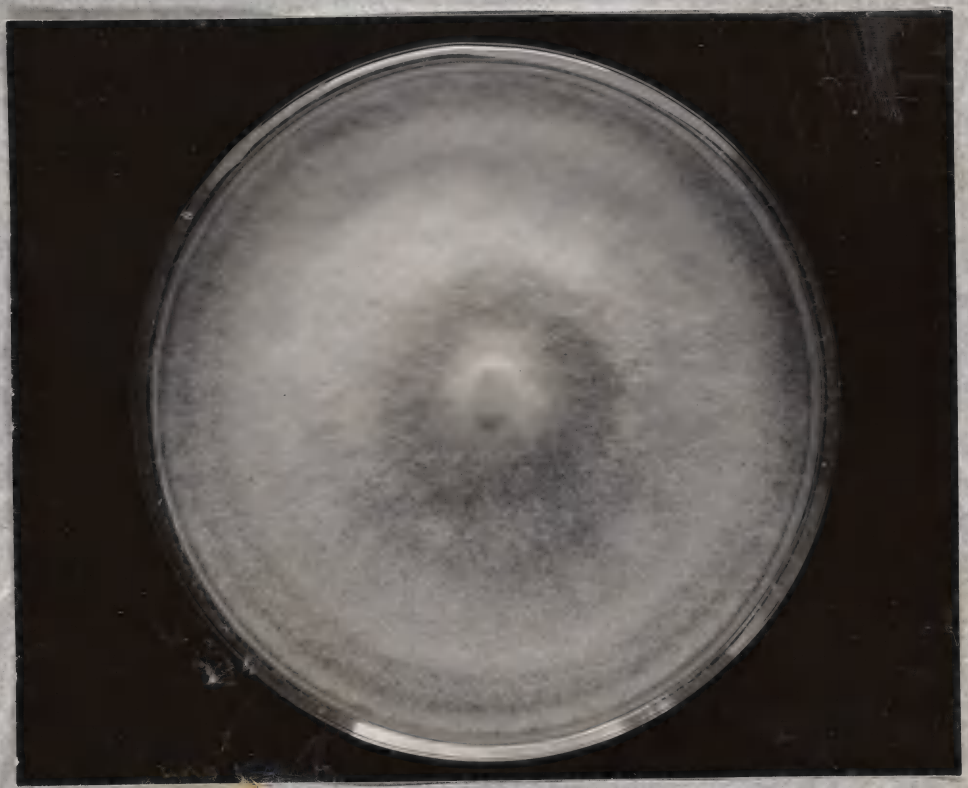

Fig. 36. Mycelial culture type of HD

Fig 30

HO - conidial culture type

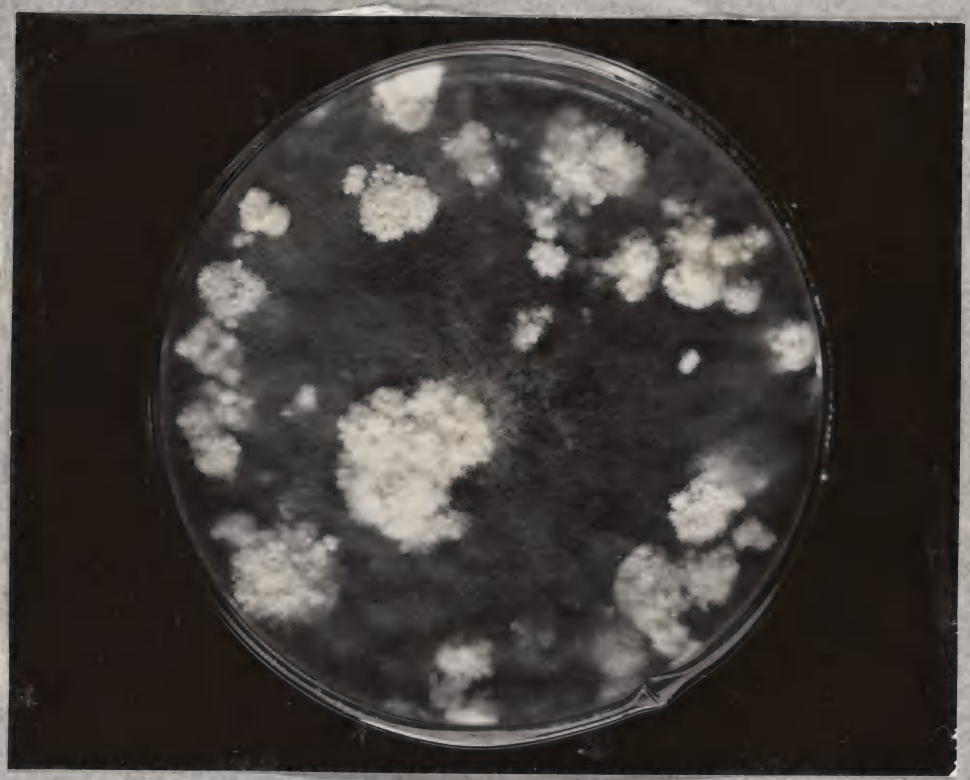

Fig. 37. Intermediate or C + M culture type of HO.

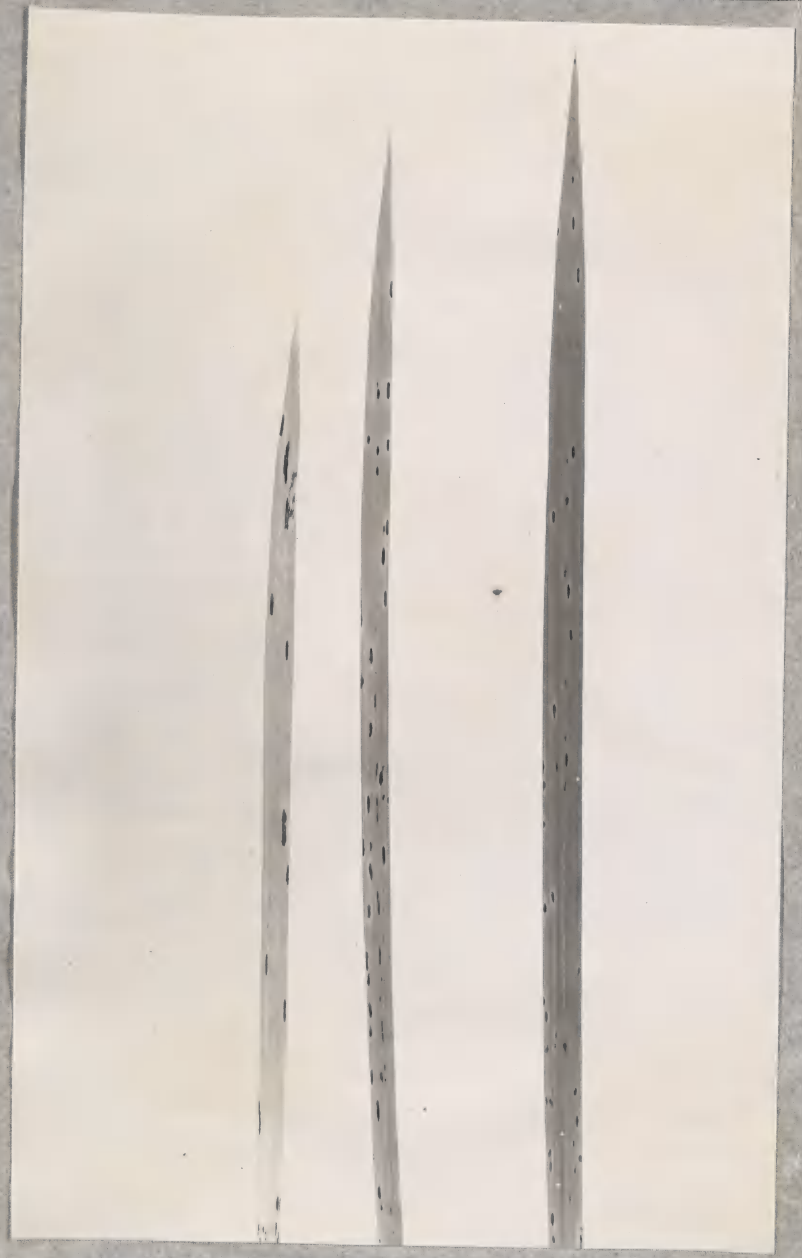

Fig. 38. MO - strain 5, spore suspension sprayed on leaves; variety Caloro.

Fig. 39. MO - strain 5, spore suspension wiped on leaves.

Figs. 38 and 39. Relative numbers of lesions of MO produced on rice by spraying spore suspension (left) vs. wiping on leaves (right).

Fig. 40. HO - strain 5
on Acadia var., sprayed.

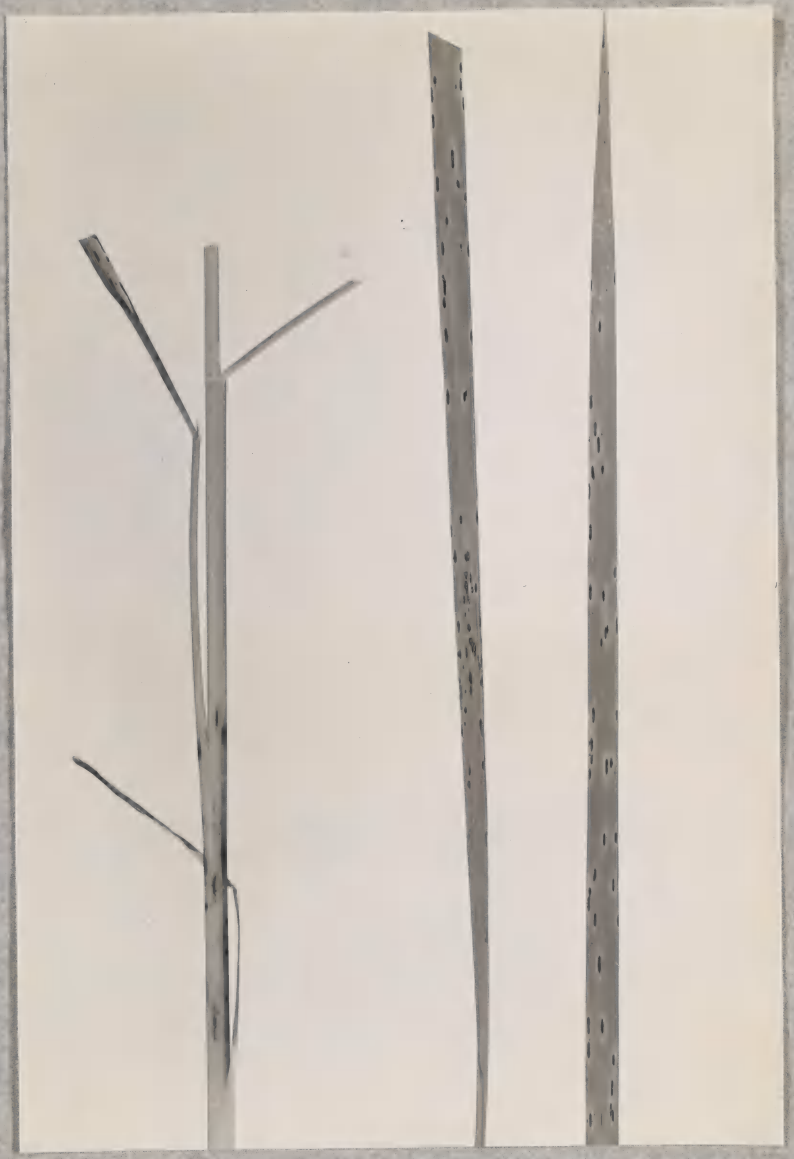

Fig. 41. HO - strain 5
on Acadia var., wiped.

Figs. 40 and 41. Relative numbers of lesions of HO produced by spraying spore suspension (left) vs. wiping on leaves and stems (right)

Fig 41

HD Spore suspension sprayed on rice leaves

Fig. 40

HD spore suspension sprayed on rice leaves

Fig. 42. RS 341 - pellet.

Fig. 43. RS 341 - sorghum seed.

Fig. 44 RS 341 + CO - pellet

Fig. 45. RS 341 + CO - sorghum seed.

Figs. 42 to 45. Results of inoculating bean plants with CO and RS.
All incubated in humidifying tent, 3 days at 25 to 30° C.

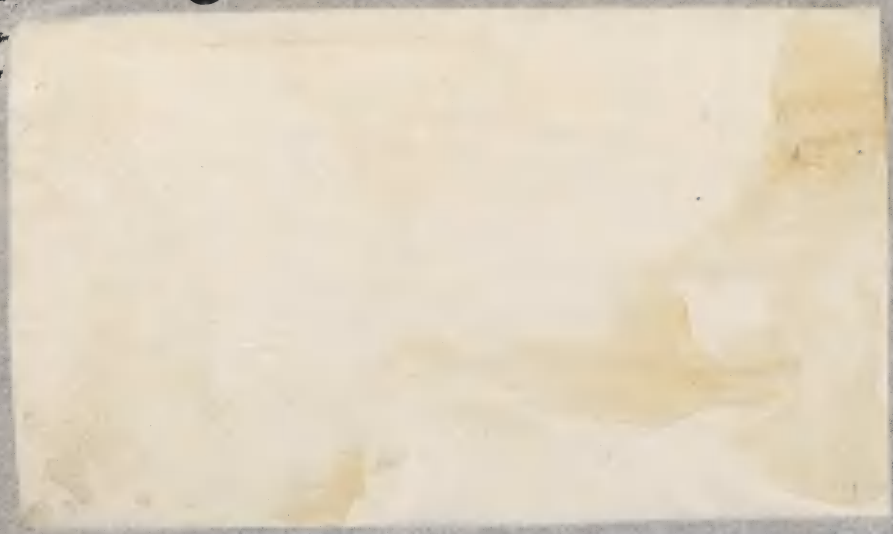

Fig. 46. CO - pellet

Fig. 47. Control plants, not inoculated.

Fig. 48. RS - potato strain + CO.

Figs 46 to 48. Results of inoculating bean plants with CO and RS, cont.


SRR - pellet